

## Genetics and Conservation of Threatened and Endangered Plant Species



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The goals of this lecture are to

- discuss the unique risks faced by plants.
- examine how genetic data have or have not been useful in Endangered Species Act decisions and actions.
- summarize the amount and nature of genetic information available for listed plants.
- make recommendations for what to do when genetic data are not available.

## Genetic Risks

- Immediate reduction in fitness
  - Inbreeding depression resulting from non-random mating
- Loss of future evolutionary potential
  - Loss of diversity
    - via genetic drift within populations
    - via loss of populations
    - via loss of species

## What's special about plants?

- Hybridization
- Sessile growth habit
- Diversity of reproductive systems
- Seed banks
- Phenotypic plasticity
  
- Lack of data
- Lack of tractable markers and gene regions

## Where Can Genetic Data be Useful?

- **Systematics**
  - General taxonomy or systematics
  - Resolving questions of evolutionary distinctiveness
- **Hybridization**
  - Identifying hybrid origin
  - Quantifying threats from hybridization
- **Within and Among Population Diversity Patterns**
  - Levels of diversity
  - Patterns of differentiation and gene flow
- **Reproductive Systems**
  - Vegetative reproduction
  - Mating system and mating patterns

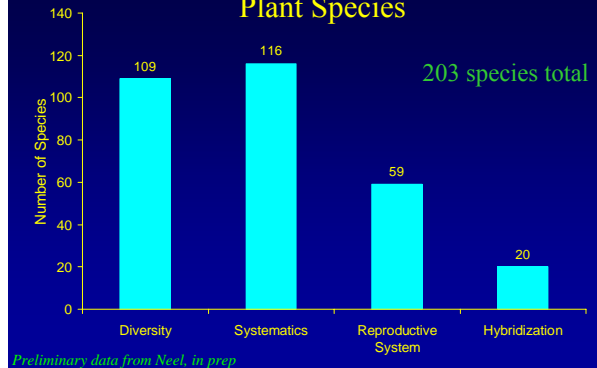
## How Many Listed Plants Have Genetic Data?

- Literature review for 748 listed plant species
  - Searched ~6,000 journals using Cambridge Scientific Abstracts' Biological Sciences, Biology Digest, and Plant Science databases in 2002 and Web of Science in 2005
  - Searched JSTOR (~60 journals) between 10/1 and 10/25 2002
  - Search terms included
    - species name for all species
    - genus name for subset of genera
    - "endangered AND plant AND genetic"
    - "threatened AND plant AND genetic"
  - Examined references cited in all approved recovery plans available online as of 10/15 2002 (n = 202 plans for 494 species)

## How Many Listed Plants Have Genetic Data?

- Resulted in
  - 304 genetics-related references
  - 264 references topics relevant to ESA
  - 203 species (27% of listed species)
- References were assigned to the four categories
  - Systematics
  - Hybridization
  - Within and Among Population Diversity Patterns
  - Reproductive System

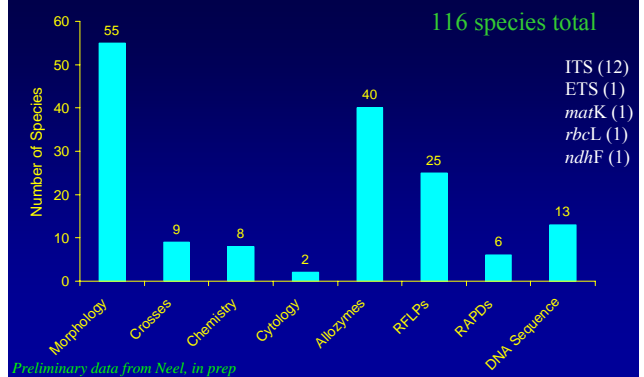
## Categories of Genetic Data for Listed Plant Species



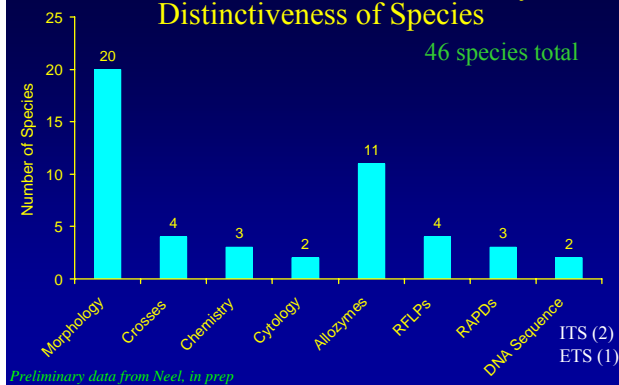
## Systematics

- Most important in listing/delisting decisions
- When assessing evolutionary distinctiveness, it is important to use both molecular and phenotypic data
  - If there is differentiation based on molecular data, you can be confident that there is reproductive isolation
  - If molecular data do not demonstrate differences, examination of other markers or phenotypic characters is warranted, especially common garden comparisons
- Regardless of the data, need to have objective criteria for determining how different is different

## Data Used in Systematics Studies



## Data Used to Assess Evolutionary Distinctiveness of Species



## Delisted Plants

- Recovered**
  - Astragalus perianus* (1978-1989)
  - Potentilla robbinsiana* (1980-2002)
  - Helianthus eggertii* (1997-2005)\*
  - Eriastrum hooveri* (1990-2003)\*
- New Information Discovered**
  - Hedeoma apiculatum* (1982-1989)
  - Tumacoca macdougalii* (1986-1993)
- Taxonomic Revision**
  - Berberis sonnei* (1979-2003)
  - Bidens cuneata* (1984-1996)
  - Echinocereus engelmannii* var. *purpureus* (1979-1989)
  - Echinocereus ×lloydii* (1979-1999)
  - Echinocereus triglochidiatus* var. *inermis* (1979-1993)



*Echinocereus X lloydii*

## Downlisted Plants

- More populations found or populations secured

*Betula uber*  
*Isotria medeoloides*  
*Mirabilis macfarlanei*  
*Pediocactus sileri*  
*Scutellaria montana*



*Scutellaria montana*

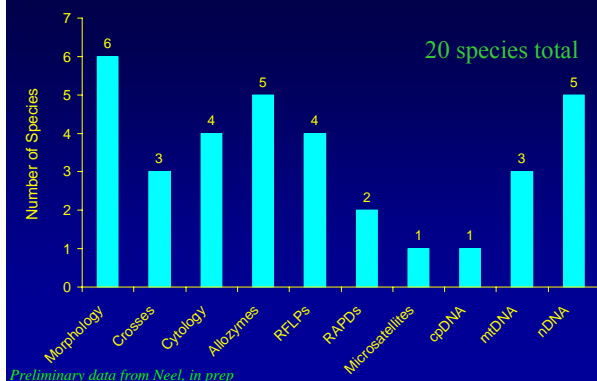
- Taxonomic merger

- *Erigeron maguirei* var. *maguirei*
  - *E. maguirei* var. *maguirei* and *E. maguirei* var. *hendersonii* merged based on RAPD data; unpublished study.

## Hybridization

- Important component of plant evolution
- Perhaps > 70 percent of plant species are descended from hybrids
- Natural interspecific common in plants and intergeneric hybridization also happens
- >1000 well-studied examples

## Data Used to Assess Hybrid Origin



## Hybridization

*Helianthus paradoxus*

*H. annuus* × *H. petiolaris*



## Hybridization

- Hybrid origin
  - Most important in listing/delisting decisions

*Echinocereus lloydii* (Lloyd's hedgehog cactus)

- Delisted due to multiple hybrid origins



Brian Fernandi

*Spiranthes diluvialis* (Ute ladies' tresses)

- Hybrid origin but is an independent evolutionary lineage with full set of chromosomes from each parent species (Arft and Ranker 1998).



Ron Shimek

## Hybridization

- Threats from hybridization most important in listing, recovery planning, and implementation

- Threat exists when
  - rare species co-occurs with a reproductively compatible related species
  - related species has larger population sizes



Roxanne Bittman

*Taraxacum californicum*  
 California dandelion

## Detecting Hybridization

- $F_1$  hybrids may have intermediate morphology – but not always.
- $F_2$ s and backcrosses may be indistinguishable from parental morphology.
- Genetic data can help – sometimes.
  - Requires codominant markers with alleles that have fixed differences between or at least very different frequencies in each of the parental species.
  - Degree of introgression will affect detection.
    - $F_{1.5}$  can be easy, later stages difficult to distinguish
  - Direction of hybridization can be detected using cpDNA and mtDNA.

## Hybridization

- *Cercocarpus traskiae*, Catalina Island Mahogany

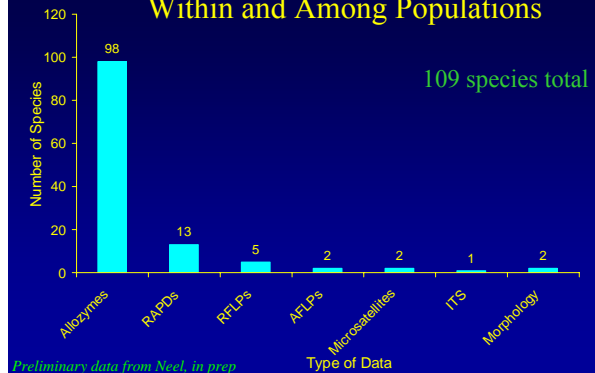


- Endemic to 1 population on Catalina Island
- 40 individuals when discovered in 1897
- 11 adults remaining in 1990s
- Exclosures to feral animals built in 1985
- 70 seedlings found after fencing
- RAPDs & allozymes indicated 5 adults and at least 5 seedlings were hybrids with *C. betuloides* var. *blanchae*
- Hybrids and common *Cercocarpus* were eliminated

## Within and Among Population Diversity Patterns

- Can potentially inform all areas of ESA actions
- Provide information on
  - Allelic richness
  - Polymorphic loci
  - Observed and expected heterozygosity
  - Inbreeding
  - Population differentiation
- Have framework for indirect comparison with other species
  - e.g., Hamrick, Linhart, and Mitton (1979) and Hamrick and Godt (1989)

## Data for Genetic Diversity Patterns Within and Among Populations



## Limitations of Diversity Surveys

- Genetic diversity will be underestimated - by some markers more than others (particularly by allozymes).
- Diversity rarely linked to fitness, threats, or management recommendations.
- Marker diversity does not necessarily reflect local adaptation.
- Lag between effects and detection of genetic differences.
- Information on gene flow patterns is indirect.
- Management recommendations might not change substantially regardless of results.

## Limitations of Diversity Surveys

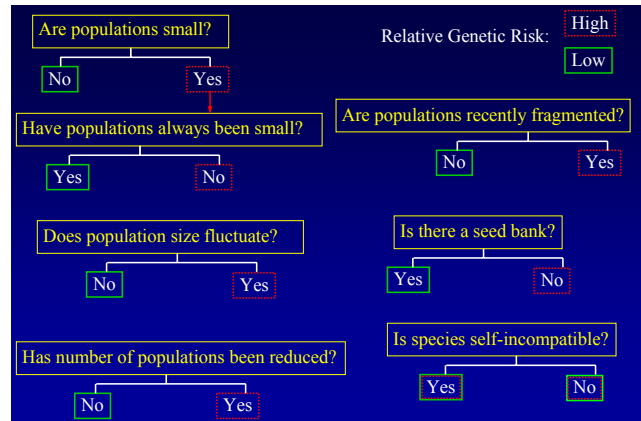
- Linking levels of diversity to fitness or to management recommendations.
  - High levels of genetic diversity and low levels of inbreeding make immediate genetic threats unlikely.
  - If not, what should you do?
- Low diversity in species and populations does not automatically imply immediate risk
  - Many plant species have always had low levels of diversity (e.g., *Howellia aquatilis*, 18 loci monomorphic)



Kirsten DuBois

## Inbreeding vs. Inbreeding Depression

- Many plants experience inbreeding without dire consequences
  - If significant inbreeding is detected, further study of inbreeding depression may be warranted
    - Compare fitness of offspring from crosses of individuals with varying levels of relatedness
  - Can also do crossing experiments to cheaply and quickly assess risk of inbreeding depression in absence of genetic analysis (e.g., *Agalinis acuta*)



There is cause for concern when



diversity  
mating patterns  
population sizes  
population proximity

have changed drastically over  
a short time period

## Diversity Changes Over Time

- Case Study: *Astragalus bibullatus*



- 7 extant & 2 extirpated populations
- Losses due to development and succession
- 4 populations sampled for allozyme diversity (Baskauf and Snapp (1998))
- Seedbank sampled from 5 populations (Morris et al. 2002)
  - Seeds from the current stratum
    - suggested more population differentiation than did older strata
    - had levels of inbreeding 10x higher than older strata but differences not significant due to large variances
  - Seed banks were more diverse than current vegetative population

## Limitations of Diversity Surveys

- Can't distinguish similarity due to shared evolutionary history from current gene flow.
  - $F_{ST}$  summarizes patterns over all populations rather than providing information on specific population pairs.
  - $F_{ST}$  only uses data from polymorphic loci thus it ignores similarities at monomorphic loci
  - Patterns can vary with different markers (cpDNA, nrDNA, and mtDNA)

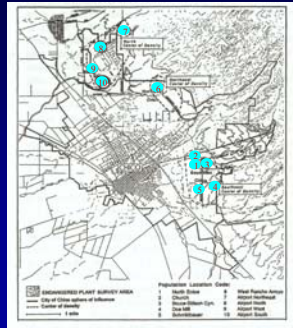
## Limitations of Diversity Surveys

- Coalescent-based approaches quantify directional gene flow among specific populations.
  - Relatively expensive data collection and sophisticated analyses
  - Typically not used in plant studies
  - Still are indirect methods

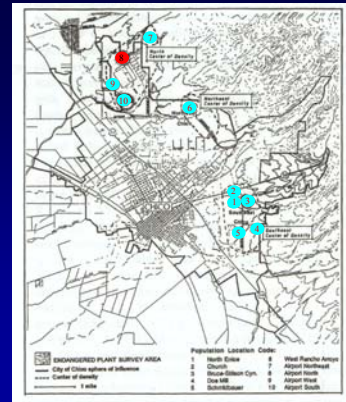


## When Genetics Can Lead You Astray

- *Limnanthes floccosa* ssp. *californica* (Dole and Sun 1992)
- Vernal pool obligate species with 11 extant occurrences

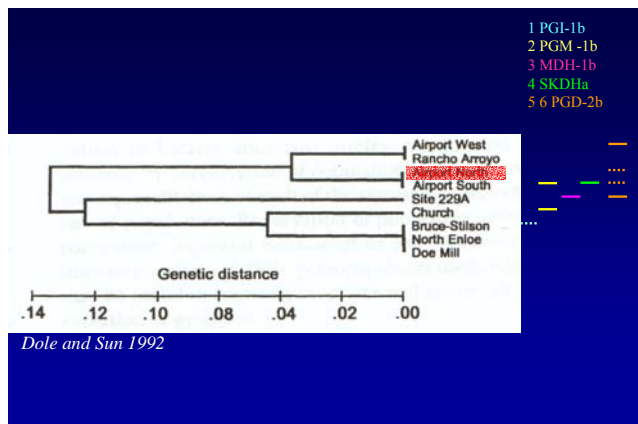


Dole and Sun 1992

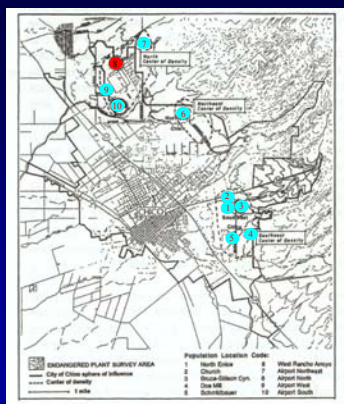


## When Genetics Can Lead You Astray

- *Limnanthes floccosa* ssp. *californica* (Dole and Sun 1992)
- Allozyme survey of 28 loci
  - 23 loci monomorphic across all populations
  - polymorphic loci had only 2 alleles each
  - see fixed differences in 4 of the 5 polymorphic loci –
  - only 3 populations polymorphic
  - $G_{ST} (\approx F_{ST}) = 0.96$



Dole and Sun 1992



Dole and Sun 1992

## Reproductive System

- Includes
  - Vegetative Reproduction
  - Mating Systems (aka Breeding Systems)
  - Mating Patterns

## Mating System

- Mating system likely is most important for assessing genetic threats and in developing recovery actions
  - e.g., translocations of compatible mating types, *Hymenoxys herbacea* (lakeside daisy)
- Mating system can be assessed relatively cheaply and quickly determined using crossing experiments



## Mating System

Questions for mating system investigations:

1. Is the species self-compatible or self-incompatible?

Pollinator exclusion and hand pollination experiments.

If self-incompatible

1. Is reproduction reduced due to lack of compatible mating types? Crosses within and among populations.

If self-compatible

1. Are pollinators required for successful reproduction? Pollinator exclusion and hand pollination comparison.
2. Are selfed matings less fit? Compare reproduction from selfed versus outcrossed matings, compare fitness of offspring.
3. What proportion of offspring are due to selfed vs. outcrossed matings? Pollinator behavior studies and genetic analysis of offspring from multiple mothers.

## Reproductive System

- e.g., *Ziziphus celata* (Godt et al. 1997, Weekley & Race 2001)
  - Five populations
  - Limited sexual reproduction
  - Self-incompatible
  - Reproduces clonally
  - All populations but one are uniclonal
  - 11 genotypes total



Jonathan Shaw © 1996

## Mating Patterns

- Primary determinant of genetic diversity patterns
- Proportion of reproduction due to selfing has large effect on rates of loss of genetic diversity over time

$$\Delta H = 1/(2N_e)$$

- Mating patterns can tell you about current population processes and potential future risks while still quantifying diversity patterns

## Mating Pattern



*Eriogonum ovalifolium* var. *vineum*, Cushenbury buckwheat (Neel et al. 2001)

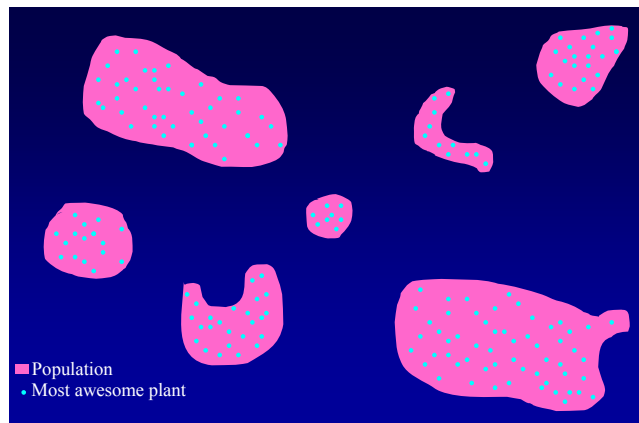
$r = 0.8$   
 $A = 2.68$   
 $H = 0.11 - 0.26$   
 $F_{ST} = 0.07$

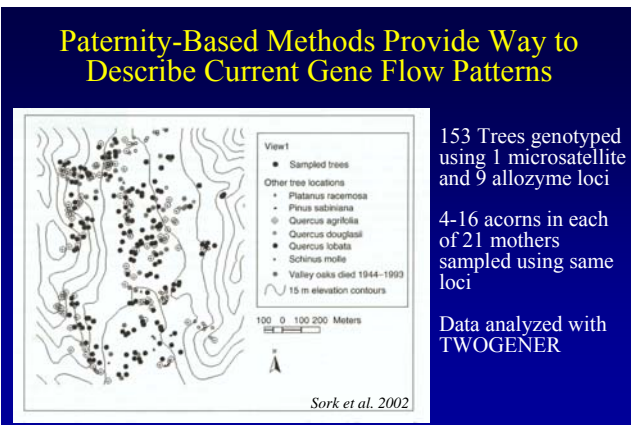
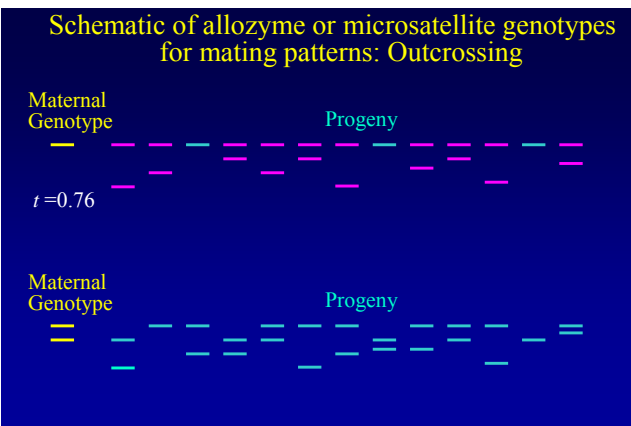
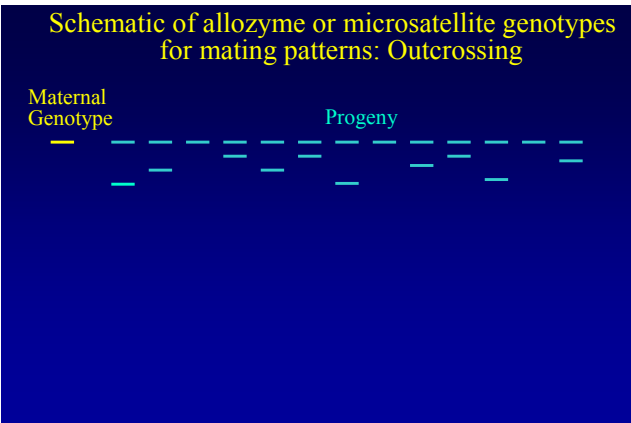
*Limnanthes floccosa* ssp. *californica*, Butte County meadowfoam (Brown and Jain 1979, Dole and Sun 1992)

$r = \text{very low}$   
 $A = 1.18$   
 $H = 0 - 0.096$   
 $F_{ST} = 0.96$

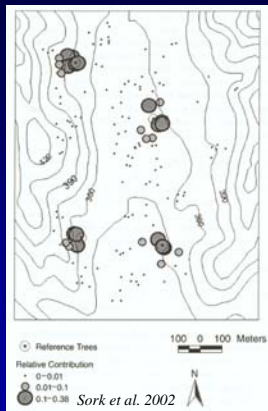


Dana Taylor









Number of effective pollen donors = 3.68  
Average distance of effective pollen movement = 64.8 m

## How Much is Enough?

Given that loss of any populations will have genetic consequences...

- How much genetic diversity within species is represented in different numbers of populations?
- How effective are ecological reserve design guidelines in representing genetic diversity?

Based on  
Neel and Cummings, 2003a & 2003b

## Suggested Conservation Intensities

How Much is Enough?

- 1 occurrence of each species (minimum sets) (e.g. Margules and Pressey 2000, Csuti et al. 1997)
- 5 populations (Brown and Briggs 1991, Center for Plant Conservation 1991, Rodrigues et al. 2000)
- 10-12% of a region or of each ecosystem in a region (World Conservation Union 1992, WWF 2001)
- 10-12% of the populations of a species (Duffy et al. 1999)
- 25-75% of an area to maintain species diversity based on island biogeography theory (Noss 1998, Soulé and Sanjayan 1998)

*Astragalus albens*



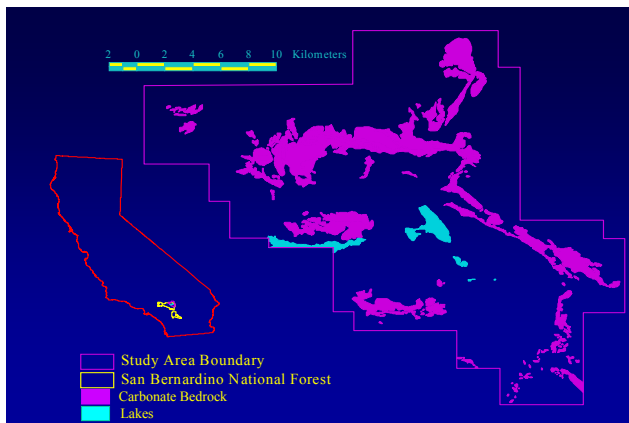
*Erigeron parishii*



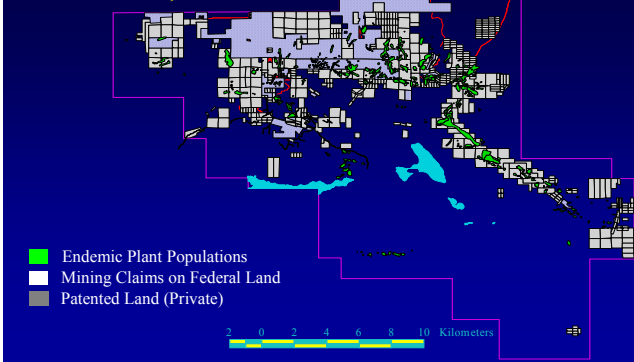
*Eriogonum ovalifolium* var. *vinetum*



*Oxytheca parishii* var. *goodmaniana*



## Mines, Mining Claims, Patented Land, & Plants



## Species Level Genetic Diversity (Allozymes)

Taxon	Pops.	Ind.	Loci	TA	CA	Theta-p
<i>Astragalus</i>	30	879	12	69	36	0.01
<i>Erigeron</i>	31	932	14	60	49	0.12
<i>Eriogonum</i>	31	929	11	60	48	0.07
<i>Oxytheca</i>	14	390	12	40	31	0.22

Neel 2000; Neel and Ellstrand 2001, accepted; Neel and Cummings in press,

TA= Total number of alleles      CA= Total number of common alleles  
Theta-p = Population subdivision

## Methods

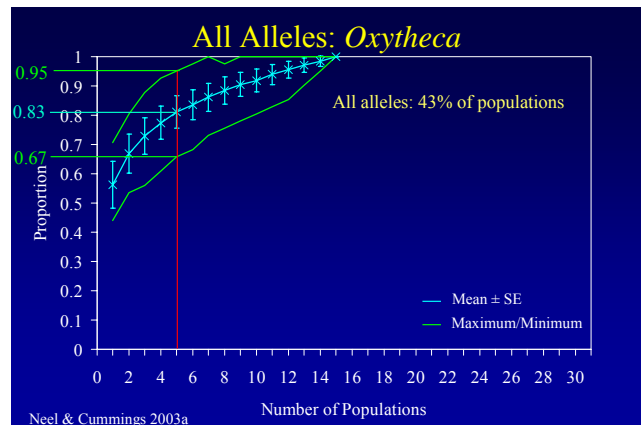
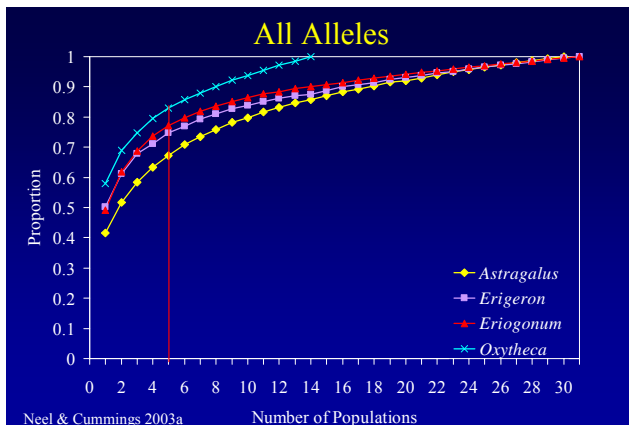
- Characterize genetic diversity in population subsets.
  - Randomly choose  $n$  populations without replacement, where  $n$  ranges from 1 to the total number of populations minus 1.
  - Calculate total number of alleles and number of common alleles for each  $n$  using the computer program GDA (Lewis and Zaykin 2001).
  - Repeat process 1000 times for each  $n$ .
  - Calculate mean, standard error, 95% confidence intervals, minimum, and maximum for all and common alleles for each  $n$ .

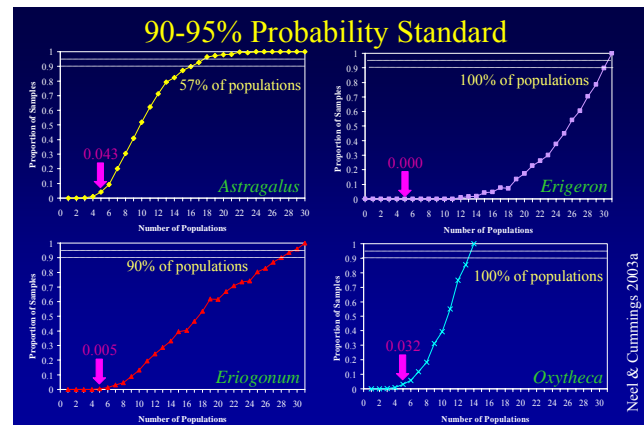
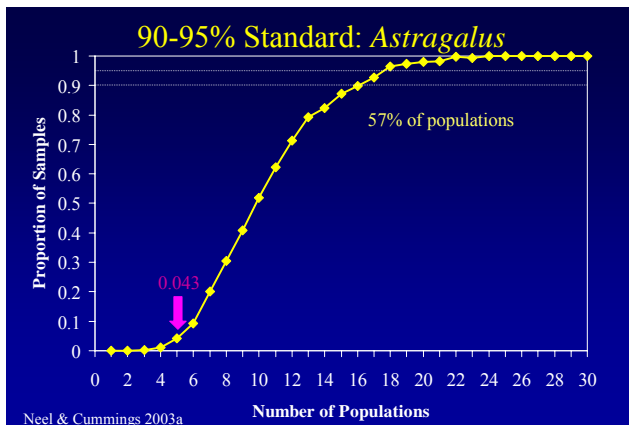
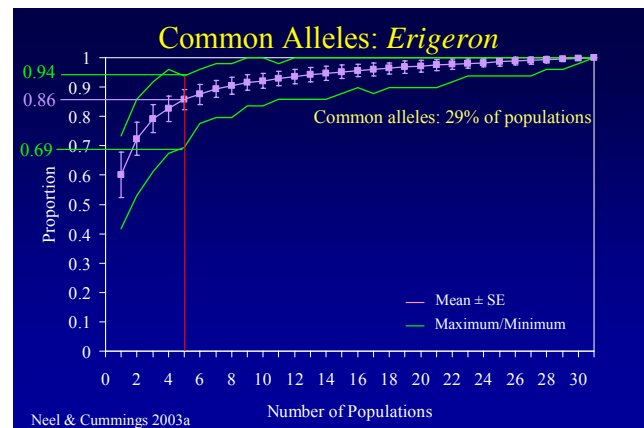
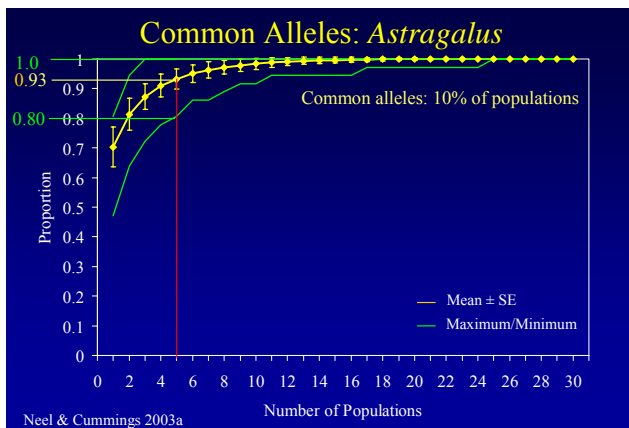
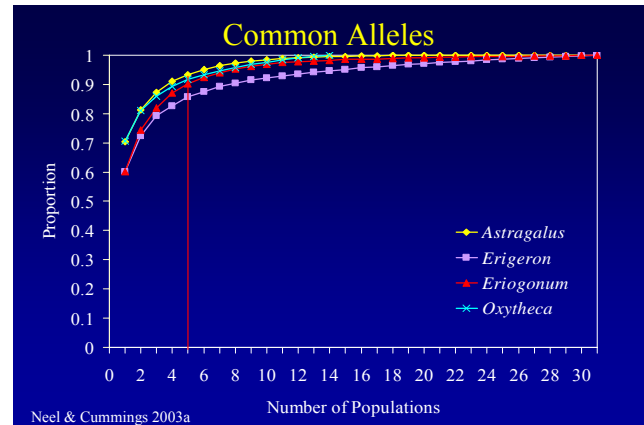
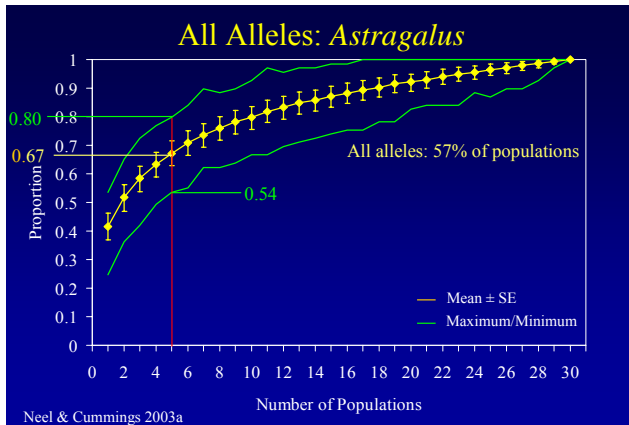
## How Much Genetic Diversity is Enough?

- Overall goals are to
  - prevent immediate deleterious effects of inbreeding.
  - maintain long-term evolutionary potential.
- Genotypic Diversity
  - All alleles to represent overall genetic diversity (Petit et al. 1998).
  - Common alleles - those at  $\geq 0.05$  frequency in populations (Marshall and Brown 1975 Brown and Briggs 1991, Center for Plant Conservation 1991).
    - Sufficient sample to have a 90-95% probability of including at least one example of all common alleles.
  - Expected heterozygosity
- Adaptive Diversity
  - ???

## Assessing Genetic Representation

- Proportion of alleles included in numbers of populations suggested in conservation targets.
- Percentage of populations needed to include all alleles.
- Percentage of populations needed to meet standard of having a 90-95% probability of including all common alleles (Center for Plant Conservation 1991).





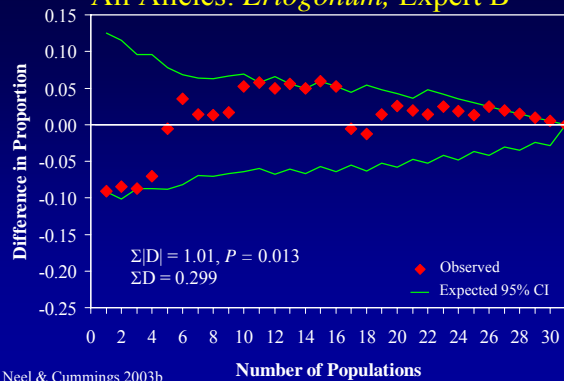
## Reserve Design Guidelines:

- Conserve largest area possible
- Conserve largest number of sites or populations possible
- Favor large populations over small
- Represent geographic and ecological range of an element
- Favor sites with more than one element
- Select defensible sites with minimal disturbance
- Minimize edge to area ratio
- Maintain historical connections between sites
- Represent range of natural features

## Effectiveness of Reserve Guidelines

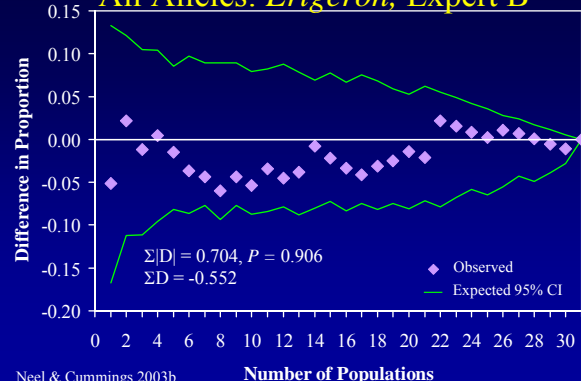
- Have four experts choose  $n$  populations according to reserve guidelines.
- Calculate diversity statistics using GDA.
- Calculate differences ( $D$ ) between random and expert selections for each expert/species/number of population combinations ( $n = 424$ ).
- Calculate sum of the absolute differences between reserve design strategies and random selections over all numbers of populations,  $\Sigma|D|$  ( $n = 16$ ).
  - Determine overall direction of differences as the sum of all differences,  $\Sigma D$ .
- Determine smallest number of populations needed for each expert to include all alleles for each species.

### All Alleles: *Eriogonum*, Expert B



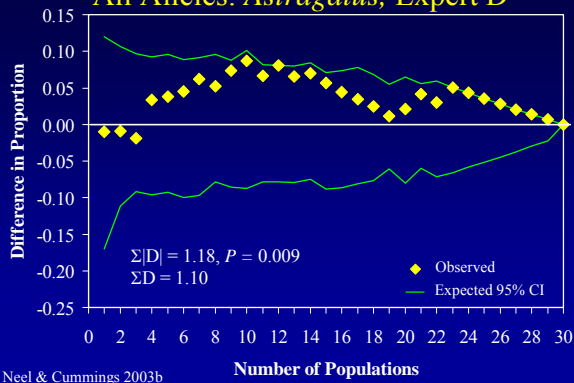
Neel & Cummings 2003b

### All Alleles: *Erigeron*, Expert B



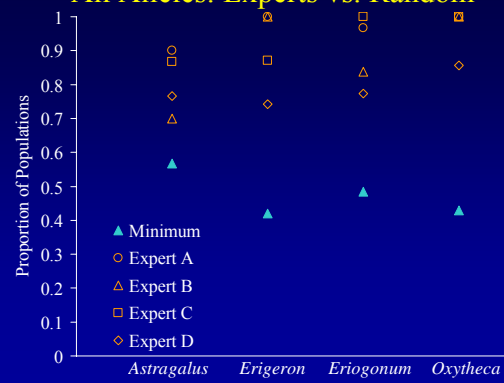
Neel & Cummings 2003b

### All Alleles: *Astragalus*, Expert D



Neel & Cummings 2003b

### All Alleles: Experts vs. Random



Neel & Cummings 2003b

### Summary: Random vs. Expert Selections

- Individual expert designs were not significantly different from random
- Some overall strategies were significantly different:
  - **All alleles:** 4 of 16 expert strategies better than expected
  - **Common alleles:** 2 expert strategies significantly worse
- Experts always required more than the minimum number of random populations to capture all common alleles
- Effects of expert strategies varied across species
- Effects of expert strategies also varied between all and common alleles in the same species

### Implications

- Genetic diversity is not necessarily easy to capture
- If capturing genetic diversity is a management goal you either need
  - large numbers or proportions of populations
  - or genetic diversity data
  - Numbers of populations needed may not be politically realistic but we should not assume genetic diversity is easy to capture
- The number of populations chosen is more important than the way they are chosen
- General reserve design guidelines have many other merits but they do not reduce the number of populations necessary to represent genetic diversity